Research update:
Microbial biodegradation of sulfolane

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Student researchers:

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Objectives: Sulfolane Biodegradation Studies

1. Determine if sulfolane degrading microbes are present in the groundwater plume, air sparge system and/or domestic water filtration systems (GAC)
2. Isolate, identify and characterize sulfolane degraders
3. Determine how sulfolane concentrations and geochemical parameters affect bacterial community structure (including any known sulfolane degraders) in the plume and air sparge system
4. Assess rates of sulfolane biodegradation by cultures and groundwater samples (plume and air sparge system)
5. Detect metabolic intermediates of sulfolane biodegradation that might provide insight into degradative pathways

Specific activities (Summer 2012): Culture-based studies

Establish and adapt previously published methods for enrichment and agar plate cultivation of sulfolane degraders in our laboratory

Isolate and identify culturable aerobic sulfolane-degrading microbes from groundwater, air sparge system and GAC (at 4° C and 23° C)

Assess the sulfolane biodegradation rates of any mixed or pure cultures obtained from groundwater, air sparge system and GAC

Develop molecular markers to detect culturable sulfolane degraders in groundwater

Culture media for sulfolane degraders

- Greene et al. 1998 developed several useful media, including:
- PAT – agar plates – a differential medium
  - **Contain sulfolane, nutrients, blue dye and some other carbon sources (dilute plate count agar - PCA)**
    - Grows sulfolane degraders, but not exclusively
      - Sulfolane degraders were found to grow best with some unknown cofactors/substrates. For this reason, a low conc. of plate count agar (PCA) included
      - Presence of PCA means that just because colonies grow, they're not necessarily sulfolane-degraders
    - PAT is a **differential medium**: contains bromthymol blue, a pH indicator
      - Sterile plates: green
      - Microbial growth present: blue
      - Sulfolane degraders present: yellow halo forms around colony (when H_2SO_4 or other acids released)

Culture media for sulfolane-degraders

We developed a new agar medium

- **SOCS** – agar plates – with sulfolane as the sole carbon source
  - **Contain sulfolane, mineral nutrients, bromthymol blue**
  - **If microbes grow, they are almost certainly sulfolane-degraders**
  - **Yellow color indicates acid production**
    - If no acid produced (no acid production or a different pathway), then sulfolane-degraders are still detected purely based on their growth on the plate.

Strategies for culturing sulfolane degraders

- Directly plate groundwater onto agar plates that are selective or differential for sulfolane degraders
  - **May detect and count sulfolane degraders if fairly abundant**
  - **Inoculated PAT and SOCS plates with:**
    - Groundwater from plume (1 ml)
    - Groundwater from air sparge (1 ml)
  - **Incubated replicates at 4˚ C and 23˚ C**
  - **PAT plates:**
    - Many colonies grew
    - No yellow color formation
    - Either too few sulfolane degraders to detect or they don't produce acid
  - **SOCS plate**
    - Little or no growth
    - Too few sulfolane degraders or they need cofactors to grow

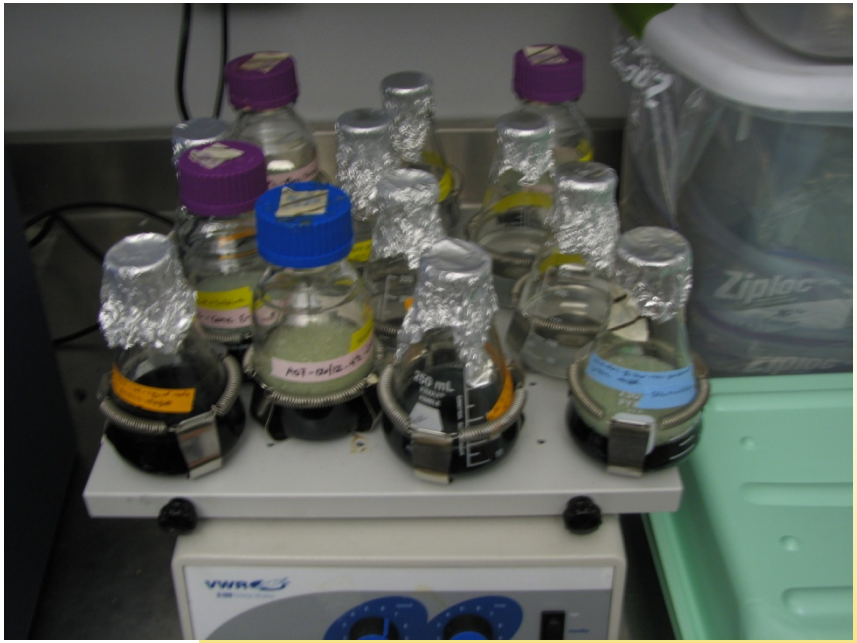
Strategies for culturing sulfolane degraders

- Create liquid enrichment cultures
  - **Favors growth of sulfolane degraders, increasing ("enriching") their numbers in a liquid medium**
  - **Common technique for detecting rare contaminant-degraders from the environment**
  - **Medium: Sulfolane as the sole carbon source**
  - **Inoculated separate enrichment cultures with:**
    - Groundwater from plume
    - Groundwater from air sparge area
    - GAC from used domestic filter
  - **Incubated on shaker for several weeks (replicates at 4° C and 23° C)**
  - **Inoculated culture onto agar plates to detect presence of sulfolane-degraders**

Enrichment Cultures – with sulfolane as sole carbon source

23°C enrichment cultures

4°C enrichment cultures



Results:

- Enrichment cultures were plated onto SOCS (sulfolane is the only carbon source available)
- All plates shown exhibit abundant bacterial growth, approx. 10^4 colonies per ml of culture
- Preliminary conclusion: Sulfolane degraders are growing in enrichment cultures.

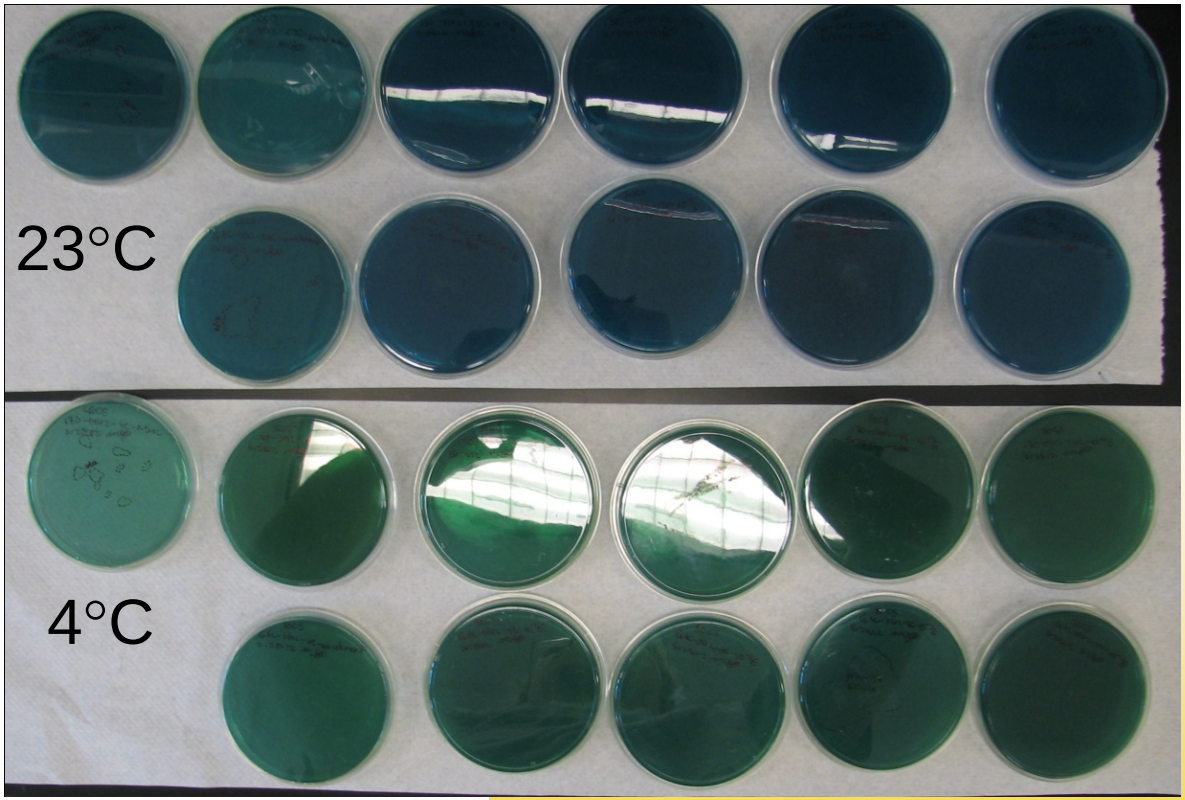
Inocula:

enrichment culture from MW-130

enrichment culture from GAC

enrichment culture from MW-130

enrichment culture from GAC



Results:

- Colonies are not forming yellow halos
- May not be releasing acid as a by-product, perhaps using different pathway, or utilizing sulfur
- May need longer incubation to show visible color change

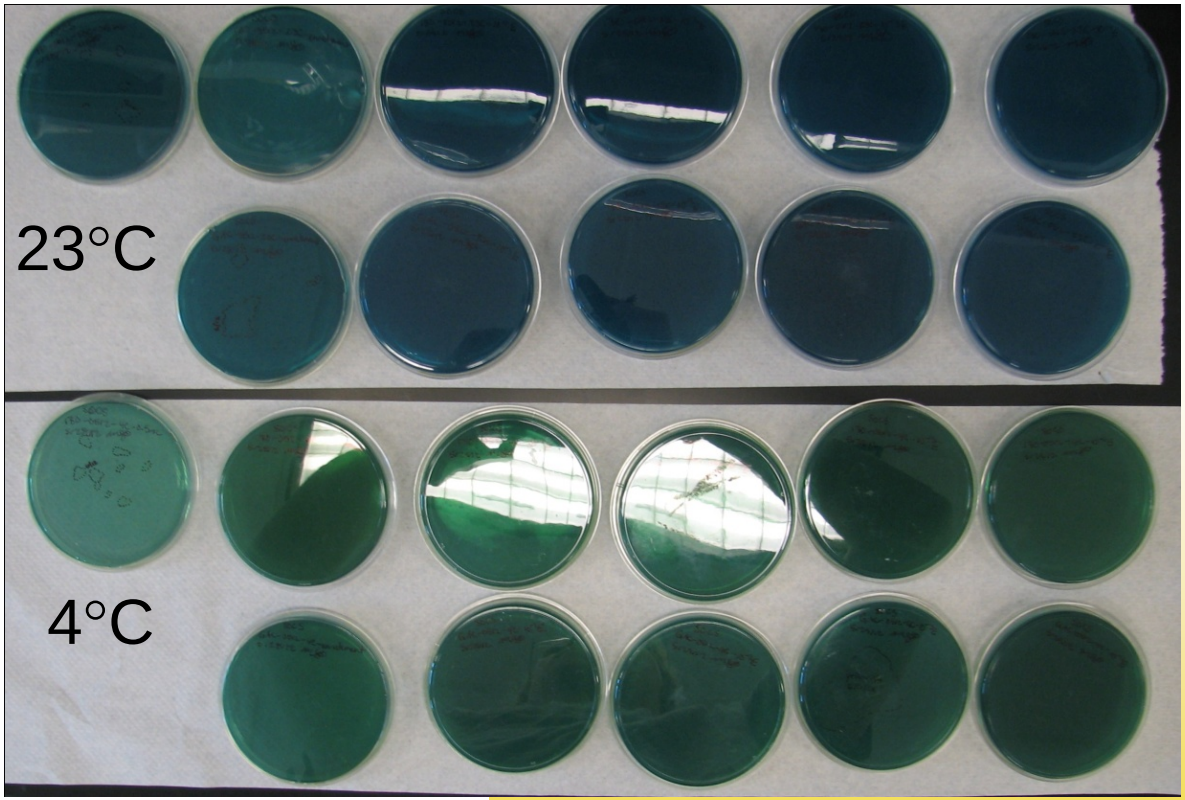
Inocula:

enrichment culture from MW-130

enrichment culture from GAC

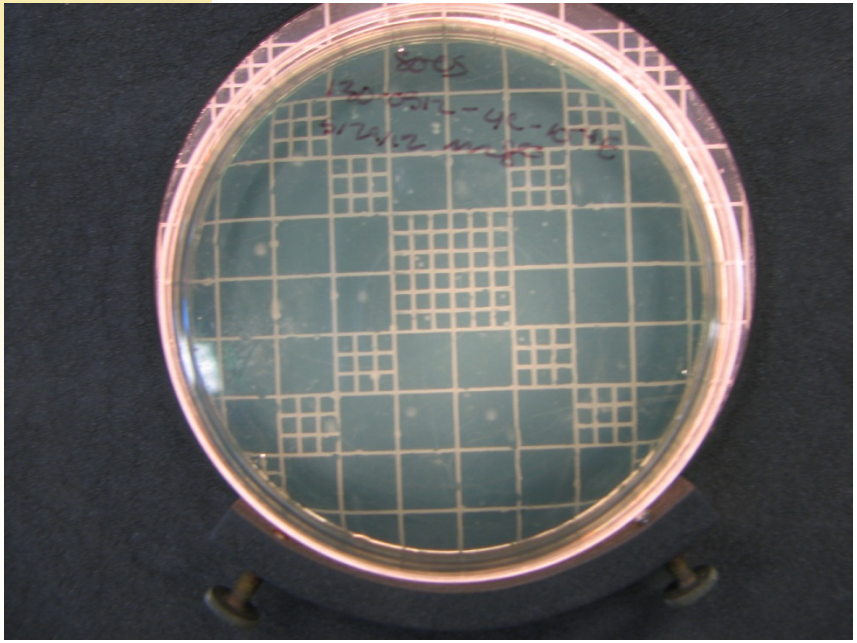
enrichment culture from MW-130

enrichment culture from GAC

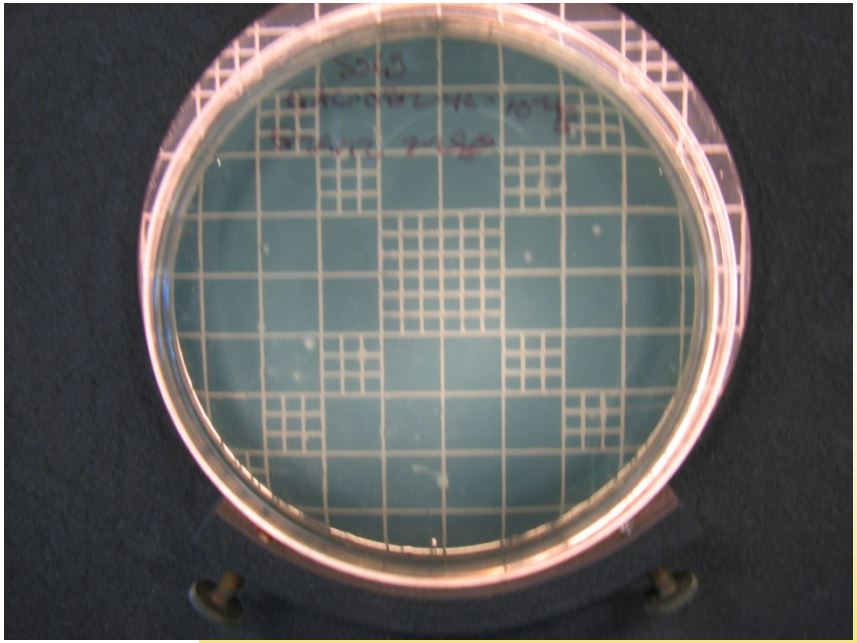


Colonies on SOCS plates

MW-130 enrichment culture, 10^{-4} dilution, 4 °C



GAC enrichment culture, 10^{-4} dilution, 4 °C



Next steps: Culture studies (in progress)

Isolate colonies into pure culture

Characterize and identify cultures

Measure sulfolane degradation rates in cultures

Look for metabolic intermediates that might provide clues to biodegradation pathway

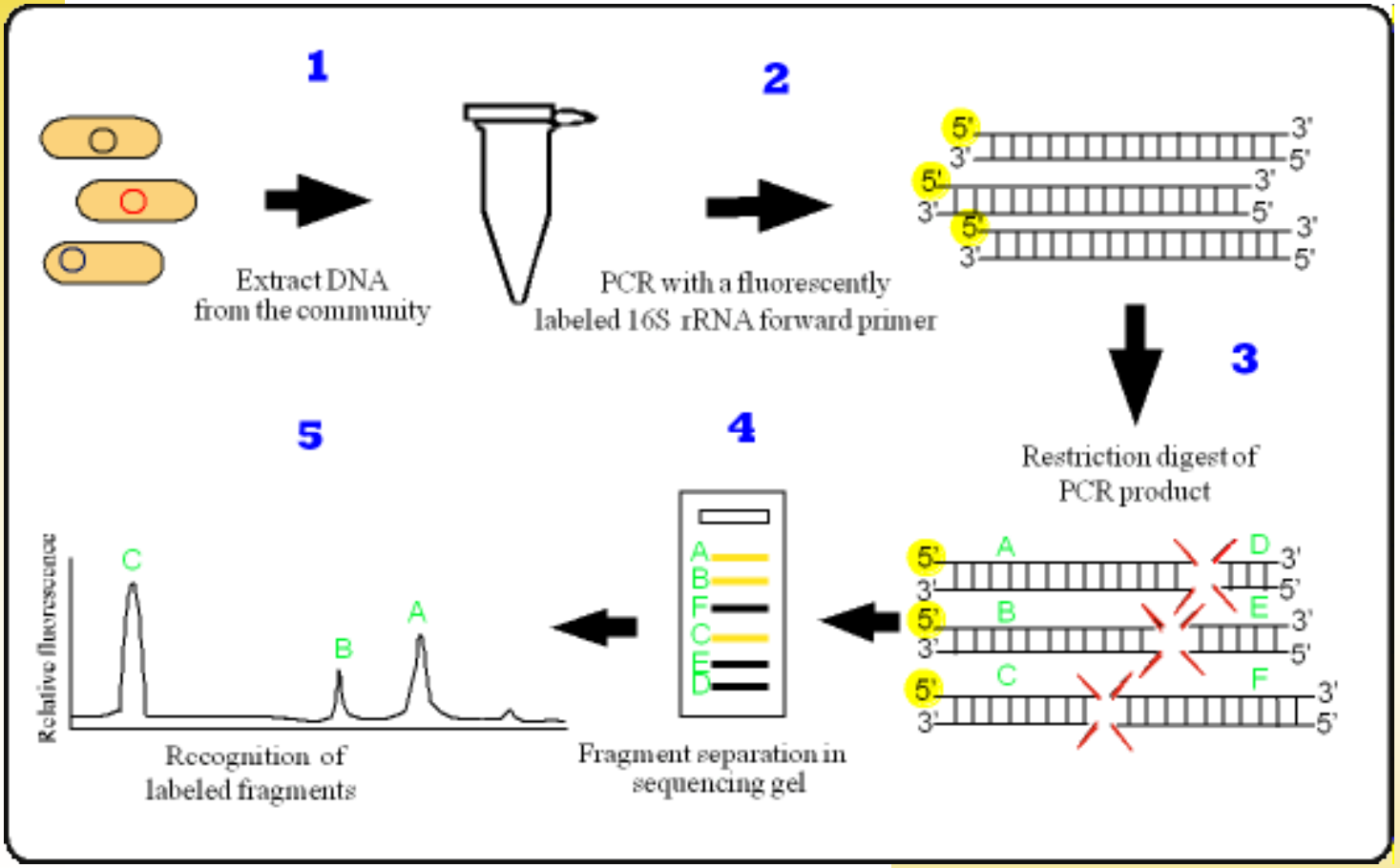
Identify molecular markers (T-RFs) for these organisms to enable direct molecular detection in plume and air sparge system

Note: analytical chemistry methods for sulfolane and metabolites now established at UAF

Specific activities (Summer 2012): Molecular characterization

- Characterize the spatial variability among bacterial communities throughout the plume and the air sparge system
- Initiate analyses of community structure variation in relation to geochemical parameters (sulfolane, petroleum, redox indicators, nutrients, etc.) throughout plume and air sparge system
- Detect sulfolane-degraders found in culture in the environment
- Initiate DNA-based stable isotope probing (DNA-SIP) studies to identify sulfolane-degraders in groundwater.

T-RFLP profiling



Marsh,et al.,1999 -Terminal restriction fragment length polymorphism (T-RFLP): an emerging method for characterizing diversity among homologous populations of amplification products. Curr. Opin. Microbiol.

Progress: Molecular characterization

- Groundwater samples provided by Shannon & Wilson
  - **Monitoring wells (those with geochemical analyses associated)**
  - **Air sparge wells(2 full sets)**
- Filtered ~1L groundwater to collect microbes
- Extracted DNA from filters
- Molecular fingerprinting using T-RFLP now in progress, should be completed in July
- Past sulfolane, petroleum and geochemical data will be provided by ARCADIS for our statistical analyses

DNA-SIP Update

- ^{13}C-labeled sulfolane just received
- Incubations of groundwater with unlabeled sulfolane will be performed to assess rates and determine appropriate incubation times
- Incubations with ^{13}C-labeled sulfolane may begin in Aug-Sept.

Stable isotope probing – Methods

Degraders incorporate ^{13}C into biomolecules

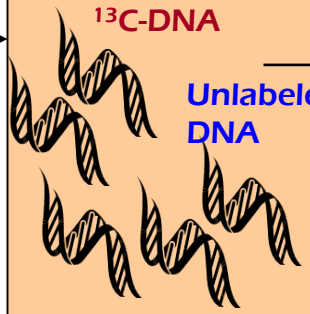
- Fatty acids (PLFAs)
- DNA
- RNA

^{13}C-labelled sulfolane

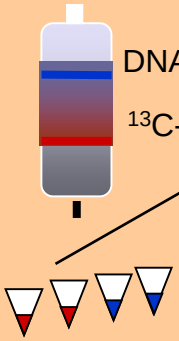
Sediment/water slurry

DNA-SIP

Extract DNA or RNA



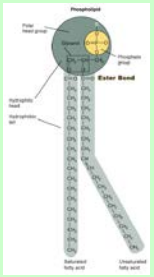
Density gradient centrifugation



Sequence 16S rRNA genes to identify microbes
- Bacteria, Archaea, others
- species-level

PLFA-SIP (will be done on BioTrap beads by Microbial Insights)

Extract PLFAs



Identify active groups (using GC-C-IRMS):

- PLFA separation (GC)
- Isotopic analysis of each PLFA (IRMS)

Highly sensitive, but only phylum-level ID

Summary

Sulfolane degrading bacteria appear be present in the plume and in GAC

Biodegradation tests will be performed on cultures to confirm this preliminary conclusion

Molecular characterization is underway and may provide insight into how abundant, variable and widespread these organisms are

Biodegradation assays of groundwater from monitoring wells and air sparge will provide additional insight into whether biological processes are contributing to sulfolane loss

Acknowledgements

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David Barnes

Shane Billings

Assistance with sampling, coordination and ^{13}C-sulfolane

Shannon & Wilson

ARCADIS

ADEC

Thanks to Jim Fish (ADEC) for delivering this update on my behalf!